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Evaluation of cold storage treatment against Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae)

In response to recent finds of live larvae in clementines (*Citrus reticulata*) that had been cold-treated during transit from Spain, a panel of ARS scientists and PPQ regulatory personnel reviewed the existing literature (see Table 1) for efficacy of cold treatment against the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae). The current treatment, T107-a, required by USDA-APHIS consists of the following temperatures and holding times:

T107-a

- 0.0°C (32°F) for 10 days
- 0.6°C (33°F) for 11 days
- 1.1°C (34°F) for 12 days
- 1.6°C (35°F) for 14 days
- 2.2°C (36°F) for 16 days

T107-a is based on the research of Hooper (1907) and Back & Pemberton (1916), which was conducted more than 80 years ago. Although there is a tendency to discount this original work because primitive equipment and techniques were used, two important facts must be considered: (1) Once a large mass of fruit is cooled to a given temperature, it tends to act as a “cold-sink” and remain at that temperature (sophisticated equipment is not necessary to maintain the temperature, just good insulation), and (2) the temperature measuring devices of that day were mercury thermometers, which are still used today, considered highly accurate, and used as the standards to calibrate thermocouples.

Work done between 1900 and 1916 was done in a pragmatic manner, and despite robust results, did not follow the systematic experimental and reporting protocols used today. The first work that was scientifically done on a large scale was in Hawaii by Back and Pemberton (1916). Back and Pemberton recommended using a treatment of 14 days at 32-33°F. This is several days longer than T-107a. Mason and McBride (1934) found that some larvae survived 31°F for 10 days. This contradicts T-107a which says that 10 days at 32°F kills all stages. Mason and McBride also found that larvae that survived a cold treatment may be moribund and die without forming puparia.. Baker (1939) using Back and Pemberton’s data estimate of the time needed to provide probit 9 using cold is 12 days at 32°F., 13 days at 33°F. and 14 days at 34°F. This is two days more than T-107a at 32°F, 33°F and 34°F. Sproul (1976) treated fruit at 0.5° for 14 rather than 11 days, he also treated fruit at 1.5°C for 16 rather than 14 days specified in T-107a.

Cold treatment research done prior to 1970 did not report complete methodology (e.g., life stage tested, age of eggs or larvae), and in some cases used too few insects for statistical analysis of probit 9 security. However, the estimated cold treatment parameters by the early researchers are close to what later researchers found. Whereas the past and

more recent evidence are complementary, it is acknowledged that some early tests did not associate quarantine security with high levels of confidence because some of the samples used were small.

Based on the scientific evidence and observations to date, the existing T-107a cold treatment, while providing a very high level of mortality, does not provide 100% mortality, and even falls short of probit 9 security. It is thus likely that the existing T-107a allows unacceptable risk of survivors when larval infestation rates are high or given slight variations in the treatment. High numbers of larvae present during the growing season may overwhelm the ability of the cold treatment to provide quarantine security. Therefore, the present cold treatment parameters are insufficient for controlling high larval populations and may result in fruit fly survivors. The level of larval infestation at which the cold treatment fails has not been determined. However, based on the literature review and all factors involved in quarantine cold treatments against Mediterranean fruit fly eggs and larvae, and given proposed recommendations to establish limits on field densities, the panel recommends increasing the length of the required treatment time at each temperature by two days. The recommended treatment parameters are:

- 0.0°C (32°F) for 12 days
- 0.6°C (33°F) for 13 days
- 1.1°C (34°F) for 14 days
- 1.6°C (35°F) for 16 days
- 2.2°C (36°F) for 18 days

The panel also recommends that long-term research plans be established by USDA-ARS and USDA-APHIS to address the issue of host effect (i.e., variation among cultivars) on fruit fly survival to verify the efficacy of the proposed new cold treatment parameters using several different citrus varieties and wild strains of Mediterranean fruit fly.

Table 1. Quarantine cold treatment references and reported treatment parameters for killing 100% of Mediterranean fruit fly eggs and/or larvae.

Reference	Temperature (°F / °C)	Life stage	Time (days)
Back & Pemberton 1916 ^a	32 / 0	Eggs ^{b, c}	11 (no data for 10 d)
	32–33 / 0–0.56	1 st instar ^c	12
	33–34 / 0.56–1.11	1 st instar ^c	16 (15 d for third instar)
	34–36 / 1.11–2.22	3 rd instar ^c	13
	36 / 2.22	3 rd instar ^c	16
	36–40 / 2.22–4.44	3 rd instar ^c	11 (no data for 10 d)
	38–40 / 3.33–4.44	3 rd instar ^c	25 (no data for 24 or 23 d)
	40–45 / 4.44–7.22	3 rd instar ^c	46
Baker 1939 ^d	32 / 0	Larvae ^e	12
	33 / 0.56	Larvae ^e	13
	34 / 1.11	Larvae ^e	14
	36 / 2.22	Larvae ^e	16
Cottier 1952	30–32 / -1.11–0	Larvae ^e	21 ^f
De Lima 1998	32.9–34.7 / 0.5–1.5	1 st instar ^c	16
Fares 1973 ^a	32 / 0	Eggs ^{b, c, g}	10
Hall 1956 ^h	31 / -0.56	Eggs & larvae ^b	21
Hill <i>et al.</i> 1988	32.9–34.7 / 0.5–1.5	Larvae ⁱ	16
Jessup <i>et al.</i> 1993	33.44–34.16 / 0.8–1.2	2 nd instar ^b	14
Hooper 1907	33–35 / 0.56–1.67	Eggs & larvae ^b	15+ (up to 21)
Lounsbury 1907	38–40 / 3.33–4.45	Eggs & larvae ^b	21–27
Mason & McBride 1934	30 / -1.11	Eggs & larvae ^b	10
	30.5 / -0.83	Eggs & larvae ^b	10
	31 / -0.56	Eggs & larvae ^b	11

Reference	Temperature (°F / °C)	Life stage	Time (days)
Nel 1936	31 / -0.56	Eggs & larvae ^b	10
	34 / 1.11	Eggs & larvae ^b	12
	37 / 2.78	Eggs & larvae ^b	16
Petty & Griffiths 1931	32 / 0	Eggs & larvae ^b	20-21
Richardson 1958 ^{j, k}	28–30 / -2.22– -1.11	Eggs & larvae ^b	5
	30–31 / -1.1– -0.56	Eggs & larvae ^b	15
Sproul 1976	32–33.8 / 0–1	Eggs & larvae	14
	34.7–35.6 / 1–2	Eggs & larvae	16

Footnotes

- a*** Low numbers of life stage(s) tested make identification of time parameters questionable.
- b*** Age of eggs and/or larvae not given.
- c*** Identified as most cold-tolerant life stage at the temperature given.
- d*** Used data from Hawaii Fruit Fly Investigations reports without references.
- e*** Larval stage not identified.
- f*** Time parameter increase to kill another insect pest in addition to Mediterranean fruit fly.
- g*** Naked larvae were used
- h*** No data given; cites New Zealand quarantine cold treatment regulations only
- i*** Larvae in confirmatory tests were 8 days old, although there was no significant differences in cold tolerance between larvae of different ages.
- j*** Cites Plant Quarantine and Control Administration Instructions 246 and 271 from the 1927 Mediterranean fruit fly outbreak in Florida; treatments specific for citrus.
- k*** Cites Bureau of Plant Quarantine Instruction 362 and Bureau of Entomology and Plant Quarantine Instruction 417; treatments specific for vinifera grapes.

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